STAINING WITH HÆMATOXYLON.

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Hæmatoxylon or logwood was first recommended by Boehmer for the staining of tissues and sections, for microscopic examinations. Its rapid action, clearness of differentiation and beautiful tint soon made it a favorite staining agent with microscopists. Possessing even a greater selective power than carmine in separating and staining the bioplasm of animal and vegetable tissues, it was also superior to this coloring agent in the fact that the violet tint of the logwood was not nearly so fatiguing to the eye in prolonged examinations with the microscope. The deeper hue of the logwood-coloring was also an advantage in the fact that the contrasts of colored and uncolored tissue afforded by its use produced a much more perfect definition and clearness of outline than could be produced by a brighter color. Nucleus, nucleolus and cell-wall, when stained by this agent, all stand out clearly and with perfect distinctness and sharpness of outline—a result not to be attained by the use of any other coloring material.

The use of logwood as a staining agent, however, was soon found to be attended with strong and serious disadvantages. The staining fluid soon became thick, cloudy and filled with a grumous sediment, at the same time changing its color; sections and tissues, stained with it, were of a dirty brown color and soon faded, and, unless the solution was freshly prepared, the results obtained from it could not be depended upon. The numerous formulæ, published by Kleinenberg, Boehmer, Miller, Klein and many others, some of which formulæ are exceedingly complicated, are sufficient proof that the task they undertook was not an easy one; and, judging by the results, a simple, satisfactory formula for preparing this desirable coloring agent has yet to be published. It is my purpose this evening to call the attention of the members of the Section to a new and simple method of preparing a logwood staining fluid, by which a permanent, reliable and satisfactory preparation can be easily made. This method, I think, will place within the reach of every microscopist a staining fluid which is stable in composition, comparatively easy of preparation and unequaled in the delicacy and clearness of differentiation of its

coloring. I have previously, at several different meetings of the Section, alluded to my experiments with this agent, and have also shown at different times some specimens of tissues stained with it; but I did not feel willing to place my results definitely before you, until sufficient time had elapsed to fully test the permanence of the preparation and of the stainings produced by its use. I have placed before you this evening, however, under the different microscopes on the table, a series of preparations, single and double stainings, which will, I think, speak for themselves, and also a sample of the staining fluid. Both fluid and specimens were prepared nearly a year ago.

In considering the method of preparation of this fluid, it will be well to review briefly the chemistry of logwood. Logwood is the heart-wood of *Hæmatoxylon Campeachianum*, a large tree found in Campeachy, Honduras and other parts of tropical America, and is used extensively in the textile arts for dyeing fabrics of a purple, blue or black color. Among its chemical constituents are resinous matter, a peculiar tannin, free acetic acid, various salts and nitrogenous principles, and a peculiar principle called hématin, or hæmatoxylon, on which the coloring properties of the wood depend. This hématin is, when pure, perfectly colorless, but affords beautiful red, blue and purple colors when in union with an alkaline base and the oxygen of the air. It also combines with the alums to form *lakes*, that peculiar class of coloring substances of which carmine is so remarkable an example. Now, this lake of logwood is the principle which acts as the dye; and, in order to obtain the color in all its delicacy and purity, all other contaminating impurities must be removed. The various formulæ for the preparation of a logwood staining fluid have nearly all directed the use of the commercial extract of logwood, which, aside from the numerous impurities necessarily found in so crude an article, is totally unfit for the purpose, for reasons which I will presently point out.

As already mentioned, logwood contains, besides its coloring principle, considerable quantities of tannin—so much, in fact, as to give it a position in the U. S. Pharmacopœia as an astringent. It is well known that vegetable infusions containing tannin are quickly influenced by the action of both light and air, and when these are assisted by heat, changes take place very rapidly. Under these circumstances, the infusions change color, become

cloudy and deposit large quantities of an insoluble sediment. It therefore can be readily understood that an extract of logwood, prepared by the evaporation of an infusion of the drug, must be to some extent changed by the process of manufacture, and that any preparation made from it would (the process of decomposition having already been started) become much more liable to change. And just such a result takes place in staining fluids prepared from extract of logwood. The partially oxidized tannin in the liquid gradually absorbs more oxygen from the air, and changes to other complex organic compounds; the coloring matter is also affected by the decomposition, and gradually becomes converted into other substances, and the liquid finally becomes of a dirty, muddy color, and is half filled with a lumpy sediment. This change will be found to take place in all ordinary logwood staining fluids, whether prepared from the extract or from the drug itself, although from the nature of the case those made from the extract would be the most quickly affected. The idea, therefore, occurred to my mind, that if the tannin could be removed, and the lake of logwood isolated in a state of comparative purity, a staining fluid could be prepared which might possibly be both permanent and satisfactory. After numerous and lengthy experiments, the desired object was obtained, and the formula which I now present to your notice is the result of my investigation on the subject. As a means of distinguishing this preparation from the other and generally worthless logwood fluids, I have thought it best to call it

"MITCHELL'S HÉMATIN STAINING FLUID."

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Finely ground logwood,	℥ ij.
Sulph. aluminum and potash (potash alum),	ʒ ix.
Glycerine,	*f.* ℥ iv.
Distilled water,	a sufficient quantity.

Moisten the ground logwood with sufficient cold water to slightly dampen it, place it in a funnel or percolator, packing it loosely, and then percolate sufficient water through the drug until the liquid coming from the percolator is but slightly colored. Allow the drug to drain thoroughly, and then remove it from the percolator, and spread out on a paper or board to dry. Dissolve the alum in eight fluid-ounces of water, moisten the dry drug with a sufficient quantity of the fluid, and again pack in the percolator, this time rather tightly, and pour on the remainder of the alum solu-

tion. As soon as the liquid percolates through and commences to drop from the end of the percolator, close the aperture with a tightly fitting cork, and allow the drug to macerate for forty-eight hours. Remove the cork at the expiration of that time, allow the liquid to drain off, and then pour sufficient water upon the drug to percolate through twelve fluid-ounces altogether. Mix this with the glycerine, filter and place in a close-stopped bottle.

In this process nearly all the tannin is removed by percolating the drug with cold water, a menstruum in which the coloring principle is not very soluble, and the subsequent maceration and percolation with the alum solution removes the logwood lake in a state of comparative purity. The glycerine is added simply for its preservative qualities, and this may be still increased by the addition of a few drachms of alcohol to the solution.

The hématin staining fluid thus prepared is a clear, heavy fluid of a deep purplish red color. It will keep its color for a length of time, and deposits no sediment. The sample exhibited to the meeting this evening has been on my working table for nearly a year, frequently exposed to a strong light and open to the air, and, as you may see, it is as yet unchanged. As a staining fluid, used either strong or diluted, I consider it far superior to any other stain I know of. Permanent and beautiful in its color, which is of a delicate violet hue, clear and sharp in its definition of the different tissues under examination, it will bear use with the very highest powers of the microscope, and, I hope, enable observers to distinguish minute differences of tissue which have hitherto escaped notice.

A few words in conclusion as regards the method of using this fluid. It yields good results, when used undiluted, as a quick stain; but the most excellent results, to my mind, are obtained by placing the tissues in a weak solution (ten drops to two fluid-drachms), with warm distilled water, for about twelve hours. This method leaves nothing to be desired, and produces results of surpassing delicacy and beauty. I had intended, in conclusion, to refer to the beautiful double-staining produced by this agent in connection with a new preparation of indigo-sulphuric acid, and have several specimens on exhibition this evening; but I think it will be best to devote a separate paper to the consideration of this subject, which I trust to be able to present at a future meeting of this Section.